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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/594,188	06/21/2007	Eugen Kolossov	2590.0040002/EJH/UWJ	7273

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EXAMINER	
CHEN, SHIN LIN	

ART UNIT	PAPER NUMBER
1632	

MAIL DATE	DELIVERY MODE
06/26/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/594,188	KOLOSSOV ET AL.	
	Examiner	Art Unit	
	Shin-Lin Chen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 March 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13,17,19-32 and 45-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13,17,19-32 and 45-70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4-14-09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' amendment filed 3-11-09 has been entered. Claims 1, 3-13, 17, 19-22, 24-27 and 29-32 have been amended. Claims 14-16, 18 and 33-44 have been canceled. Claims 45-70 have been added. Claims 1-13, 17, 19-32 and 45-70 are pending and under consideration.

Information Disclosure Statement

1. The information disclosure statement (IDS) submitted on 4-14-09 was filed after the mailing date of the non-final Official action on 12-11-08. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 1-13, 17, 19-32 and 45-70 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants' amendment filed 3-11-09 necessitates this new ground of rejection.

The phrase "wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml" in amended claim 1 is considered new matter. Applicants point out support for said amendment at page 10, lines 20-23, page 11, lines 20-30, in Example 1 at page 42, lines 23-26 and in Example 3 and 4 at page 43, line 29, and at page 44, line 15 of the specification. The cited support only discloses 1 to $5x10^6$ cells/ml or $0.1\text{-}0.5x10^6$ cells/ml. There is no support for "a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml". Thus, the phrase "wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml" is considered new matter. Claims 2-13, 17, 19-32 and 69 depend from claim 1.

The phrase "wherein said culture of pluripotent cells has a concentration of about $0.1x10^6$ to $1x10^6$ cells/ml" in newly added claim 45 is considered new matter. Applicants point out support for said amendment at page 10, lines 32 to page 11, line 2 and lines 22-30, original claim 1, in Example 1 at page 42, lines 23-26 and in Example 2 at page 43, line 14 of the specification. The cited support only discloses 1 to $5x10^6$ cells/ml or $0.1\text{-}0.5x10^6$ cells/ml. There is no support for "a concentration of about $0.1x10^6$ to $1x10^6$ cells/ml". Thus, the phrase "wherein said culture of pluripotent cells has a concentration of about $0.1x10^6$ to $1x10^6$ cells/ml" is considered new matter.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-3, 7-13, 17, 31, 32, 45-47, 51-54, 67 and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) or Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS) each in view of Yan et al., February 2003 (US 20030027331 A1) and Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS). Applicants' amendment filed 3-11-09 necessitates this new ground of rejection.

Claims 1-3, 7-13, 17, 31, 32, 45-47, 51-54, 67 and 68 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid single cell suspension culture of pluripotent cells in a container until generation of cell aggregates and diluting the suspension and further agitation of the suspension until formation of EBs, wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, wherein the cells could be cultured on embryonic mouse fibroblasts feeder cells before agitation of suspension culture, an embryoid body obtained from said method, and cardiomyocytes or tissue of cardiomyocytes obtained from said embryoid body. Claim 7 specifies the concentration of pluripotent cells is

about $1x10^6$ to $5x10^6$ cells/ml. Claims 8-10 specify the suspension is cultured for about 6 hours, 16 to 20 hours and in T25 flasks, respectively. Claims 11 and 12 specify the dilution is 1:10 and the final concentration of EBs in the suspension culture is about 500/ml, respectively. Claims 51 and 52 specify the culture of pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, respectively. Claim 53 specifies the EBs are diluted to about 100-2000 EBs/10ml. Claims 17 and 54 specify the cells are differentiated into cardipmyocytes.

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryonic stem cells by removing the adhering colonies of the embryonic stem cells from the substrate in clumps and then incubating the clumps in a container under conditions that essentially inhibit the clumps from attaching to the container and under conditions in which the clumps of embryonic stem cells coalesce into embryoid bodies (e.g. claim 1). Thomson teaches culturing primate embryonic stem cells, such as rhesus or human ES cells, on inactivated mouse embryonic fibroblasts with culture medium DMED and 20% FBS and the ES cell colonies are removed from the tissue culture plate using physical or chemical methods, such as dispase or collagenase (e.g. column 3, lines 48-67). Thomson further teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension. Cell suspension s are aliquoted into wells of 6-well tissue culture dishes, placed inside a sealed, humidified isolation chamber, gassed with 5% CO2, 5% O2, and 90% N2 and placed on a rocker … The rocker is housed inside an incubator maintained at 37oC. The culture plates can be rocked continuously for at least 48 hours and up to 14 days" (e.g. column 4, 3rd full

paragraph). Rocking suspension culture on a rocker is a type of agitation of the suspension culture.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Some ES cell lines require aggregation of multiple ES cells to enable EB formation and the EB can be further differentiated into differentiated embryonic stem cells and/or tissue, such as cardiomyocytes or cardiac tissue (e.g. [0020]). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation via stirring or agitation of the liquid suspension (e.g. [0051], [0053]).

Thomson and Dang do not specifically teach growing single cell suspension of the pluripotent cells or the cell concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr.

Yan discloses that homozygous stem (HS) cells are pluripotent cells (e.g. [0016]) and teaches trypsinizing the HS cells grow the HS cells in single cell suspension culture in 2 ml ES-LIF medium and culturing the cells as suspension cells in suspension culture at a density of $1-3x10^6$ cells to allow stem cells to form rounded spherical clusters known as embryoid bodies (EBs) for 4-6 days (e.g. [0293]). The isolated HS cells can be induced to differentiated into cardiomyocytes using technique known in the art such as Kehat (2001) (e.g. [0220]).

Kehat teaches culturing human ES cells in suspension and plated to form EBs, and spontaneously contracting areas appeared in 8.1% of EBs. Cells from said contracting area

within EBs were stained positively with anti-cardiac myosin heavy chain, anti-alpha-actinin, anti-desmin, anti-cardiac troponin 1 and anti-ANP antibodies. The human ES cell-derived cardiomyocytes displayed structural and functional properties of early stage cardiomyocytes (e.g. abstract). ES cell clumps were grown in plastic petri dishes at a cell density of about $5x10^6$ cells in a 58 mm dish (e.g. p. 408, bridging left and right column).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to grow pluripotent cells in single cell suspension because Yan teaches growing HS cells, which are pluripotent cells, in single cell suspension culture to from embryoid bodies. It also would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to culture the ES cells at a concentration of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, with a certain final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr because Thomson teaches culturing the ES cells for at least 48 hours and up to 14 days, Yan teaches growing pluripotent cells at a concentration of $1\text{-}3x10^6$ cells/2ml and Kehat teaches culturing ES cells at about $5x10^6$ cells in a 58 mm dish. The concentration of $1\text{-}3x10^6$ cells/2ml is within the range of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Culturing ES cells at these conditions or with a certain final concentration of EBs would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson, to form

EBs from HS cells as taught by Yan or to form EBs from human ES cells as taught by Kehat with reasonable expectation of success.

Applicants argue that Thomson does not teach single cell suspension culture of pluripotent cells to form EBs. Applicants further argue that Thomson teaches using the rocking table not for generation of aggregates themselves but rather only to prevent the already existing aggregates from sticking to surface during culture and Thomson does not teach a cell concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml (amendment, p. 18-20). Applicants also argue that Kehat does not teach single cell suspension culture and agitation to produce aggregates and EBs (amendment, p. 25). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection. Yan teaches growing HS cells, which are pluripotent cells, in single cell suspension culture to from embryoid bodies. Yan also teaches growing pluripotent cells at a concentration of $1\text{-}3x10^6$ cells/2ml, which is within the range of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Further, determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Thomson teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension". Thus, the gently and continuously rocking the cell suspension is to maintain the cell suspension and also for **further embryoid body formation**. Rocking the cells also provide the formation of embryoid body.

Applicants argue that Dang does not teach agitation for controlling aggregation of EBs rather Dang teaches agitation as an alternative of stirring to keep cells and/or spheroids in liquid suspension (amendment, p. 20-21). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection. Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation via stirring or agitation of the liquid suspension (e.g. [0051], [0053]). Dang teaches that "the invention provides a method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled. In yet another embodiment, the invention provides a scalable and controllable culture of spheroid-forming cells, such as pluripotent cells by allowing them to be cultured in stirred bioreactors, such as stirred liquid bioreactors. This is done by controlling cell aggregation." (e.g. [0052]). It is apparent that stirring or agitation is for controlling cell suspension culture and for controlling cell aggregation, i.e. forming EBs.

7. Claims 1-6 and 45-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) or Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS) each in view of Yan et al., February 2003 (US 20030027331 A1) and Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS) as applied to claims 1-3, 7-13, 17, 31, 32, 45-47, 51-54, 67 and 68 above, and further in view of Dang et al., 2002 (Biotechnol Bioeng, Vol. 78, p. 442-453). Applicants' amendment filed 3-11-09 necessitates this new ground of rejection.

Claims 1-6 and 45-50 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid single cell suspension culture of pluripotent cells in a container until generation of cell aggregates and diluting the suspension and further agitation of the suspension until formation of EBs, wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Claims 4 and 48 specify the cells are obtained from a murine ES cell line. Claims 5 and 49 specify the culture medium is IMDM, 20% FCS and 5% CO2. Claims 6 and 50 specify the culture condition comprises 37oC and 95% humidity.

The teachings of Thomson, Dang (2003), Yan and Kehat are as discussed above. Thomson, Dang (2003), Yan and Kehat do not specifically teach using murine ES cells, IMDM culture medium, 20% FCS and 95% humidity.

Dang (2002) teaches culturing CCE murine embryonic stem cell in IMDM medium at 37oC in humidified air with 5% CO2 (e.g. p. 444, left column, Materials and Methods). Dang use liquid suspension cultures of murine ES cells to form EBs (e.g. p. 444, right column, 1st paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to culture murine ES cells in IMDM medium at 37oC in humidified air with 5% CO2 because Dang teaches culturing murine ES cells in said condition. It also would have been prima facie obvious for one of ordinary skill in the art to culture ES cells in medium having 20% FCS and 95% humidity because Thomson teaches culturing ES cells in medium having 20% FBS and both Thomson and Dang teach culturing ES cells in humidified condition. FBS and FCS are the same and growing ES cells in 95% humidity would be obvious to one of ordinary

skill in order to optimize the culture condition for the ES cells. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson or to form EBs from murine ES cells as taught by Dang with reasonable expectation of success.

Applicants argue that Thomson and Dang (2002) in combination would not arrive at the instant claimed invention that uses agitation of a liquid single cell suspension culture of pluripotent cells to generate cell aggregates and EBs. Dang does not teach or suggest that the liquid suspension culture should be agitated to generate cell aggregates and EBs, and Dang does not teach the pluripotent cells at a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml (amendment, p. 21-23). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection. Yan teaches growing HS cells, which are pluripotent cells, in single cell suspension culture to from embryoid bodies. Yan also teaches growing pluripotent cells at a concentration of $1\text{-}3x10^6$ cells/2ml, which is within the range of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Further, determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Thomson teaches "[o]nce colonies are removed from the tissue culture plate, the ES cells should remain in suspension during further embyoid body formation. This can be achieved by, for example, gently and continuously rocking the cell suspension". Thus, the gently and continuously rocking the cell suspension is to maintain the cell suspension

and also for **further embryoid body formation**. Rocking the cells also provide the formation of embryoid body.

8. Claims 1, 17, 19-30, 54-66, 69 and 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al., 2003 (US Patent No. 6,602,711 B1) in view of Yan et al., February 2003 (US 20030027331 A1) and Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS) as applied to claims 1-3, 7-13, 17, 31, 32, 45-47, 51-54, 67 and 68 above, and further in view of Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS). Applicants' amendment filed 3-11-09 necessitates this new ground of rejection.

Claims 1, 17, 19-30, 54-66, 69 and 70 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as embryonic stem cells, comprising agitation of a liquid single cell suspension culture of pluripotent cells in a container until generation of cell aggregates and diluting the suspension and further agitation of the suspension until formation of EBs, wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml. Claims 17 and 54 specify the EBs are allowed to differentiated into cardiomyocytes. Claims 19-30 and 55-66 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 27 and 28 specify the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule and on the same cistron, respectively. Claims 69 and 70 specify the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v, respectively.

The teachings of Thomson, Yan and Kehat are as discussed above. Thomson, Yan and Kehat do not specifically teach using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence, the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule or on the same cistron.

Dang (2003) teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Some ES cell lines require aggregation of multiple ES cells to enable EB formation and the EB can be further differentiated into differentiated embryonic stem cells and/or tissue, such as cardiomyocytes or cardiac tissue (e.g. [0020]). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation via stirring or agitation of the liquid suspension (e.g. [0051], [0053]). Dan also teaches individual R1 ES cells expressing GFP protein under the control of a constitutively active promoter was encapsulated with a cyan labeled ES cell, and the two cell types survived and proliferated to form spheroid containing two sources of cells (chimeric spheroids). The technology of forming chimeric spheroids can be used to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals (e.g. [0185]).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to prepare ES cells expressing a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence because Dang teaches using a ES cell expressing GFP under the control of

constitutively active promoter and GFP is a type of selectable marker, and one of ordinary skill would use another selectable marker or a cell specific promoter in order to optimize the expression of the marker at target cells and to optimize visualization or detection of the ES cells. Having the marker gene and reporter gene contained on the same recombinant nucleic acid molecule or on the same cistron would be obvious to one of ordinary skill because determining effective orientation of the marker gene and reporter gene is routine optimization of a result-effective variable and is obvious to one of ordinary skill. One of ordinary skill would orient the marker gene and reporter gene in a vector in order to optimize the expression of the marker gene and reporter gene at target cells.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from primate ES cells as taught by Thomson or to form chimeric spheroids to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals as taught by Dang with reasonable expectation of success.

Applicants argue that neither Thomson nor Dang teach agitating a liquid single cell suspension culture to generate aggregates and EBs. The rocking table as taught by Thomson is not for generation of aggregates rather only to prevent the already existing aggregates from sticking to the surface during culture. Dang suggests agitation as a method to keep individual cells and/or spheroid in liquid suspension (amendment, p. 26-27). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection.

Conclusion

No claim is allowed.

9. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shin-Lin Chen whose telephone number is (571) 272-0726. The examiner can normally be reached on Monday to Friday from 9:30 am to 6 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for this group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Shin-Lin Chen, Ph.D.
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Primary Examiner, Art Unit 1632